#### 5328us.ST25.txt SEQUENCE LISTING

```
<110> Sobek, Harald
      Frey, Bruno
      Antranikian, Garabed
      Boehlke, Kristina
      Pisani, Francesca Maria
      Rossi, Mose
<120> Mutant B-type DNA Polymerases Exhibiting Improved Performance in PCR
<130>
       5328
. <150>
       EP/00105155.6
<151>
       2000-03-11
       34
<160>
<170> PatentIn version 3.0
 210>
       1
 211>
       35
       DNA
       Artificial
       amplification primer
                                                                         35
dcaaccttgt agagtagagt ggtacctgtt aaggg
 <210>
       2
  711>
       44
 <212>
       DNA
<213>
       Artificial
<220>
<223>
       amplification primer
<400>
                                                                         44
gcctctttcc ggctctttta cgtatcctcc caggaaagta gtcc
<210>
        3
<211> 44
<212>
      DNA
```

<213> Artificial

```
<220>
<223>
       amplification primer
<400>
       3
                                                                          44
gcctctttcc ggctctttta cgtatcctcc caggtgagta gtcc
<210>
<211>
       44
<212>
       DNA
<213>
       Artificial
<220>
<223> amplification primer
<400>
                                                                          44
gcctctttcc ggctctttta cgtatcctcc caggttagta gtcc
       5
211>
       44
       DNA
       Artificial
       amplification primer
gcctctttcc ggctctttta cgtatcctcc cagggaagta gtcc
                                                                          44
       6
       44
<212>
       DNA
<213>
       Artificial
<220>
<223>
       amplification primer
<400>
gcctctttcc ggctctttta cgtatcctcc cagccaagta gtcc
                                                                          44
<210>
       7
<211>
       44
<212>
       DNA
<213>
       Artificial
```

<220> <22 <sup>3</sup> >	amplification primer	
<400>	7	44
geetett	ttcc ggctctttta cgtatccagc caggtaagta gtcc	44
<210>	8	
<211>	34	
<212>	DNA	
<213>	Artificial	•
<220>		
<223>	amplification primer	
<400>	8	2.4
agcttat	cga tggcactttt cggggaaatg tgcg	34
<210>	9	
2117	36	
₹112>	DNA	
T-1-	Artificial	
< <u>2</u> 20>		
<220> <223>	amplification primer	
< <u>4</u> 00>	9	
24.45	cga taagcggatg ccgggagcag acaagc	36
L)		
<210>	10	
<211>	23	
<212>	DNA	
<213>	Artificial	
<220>		
<223>	amplification primer	
<400>	10	
gatgagt	ttog tgtccgtaca aca	23
<210>	11	
<211>	23	
<212>	DNA Artificial	
<213>	Artificial	

<220> <223>	amplification primer	
<400>	11	
		2.2
CLCalc	agca gatcatcttc agg	23
<210>	12	
<211>	23	
<212>	DNA .	
<213>	Artificial	
<220>		
<223>	amplification primer	
<400>	12	
actcca	gcgt ctcatcttta tgc	23
210> 211> 212> 212> 213>		
<210>	13	
₹211>	23	
<b>₹</b> 212>	DNA	
∢213>	Artificial	
₹220> ₹223>		
∢223>	amplification primer	
±400>	13	
gatggt	gatc ctctctcgtt tgc	23
(210> <211> <212>		
√ <u>0</u> 10\	1.4	
\210\\ \211\\	14	
(212)	23 DNA	
<212>		
<213>	Artificial	
<220>		
	amplification primer	
<400>		
gatgag	ttcg tgtccgtaca aca	23
Z0105	15	
<210>		
<211>		
<212>	DNA	
2 1 1 1 1 X	0 x + 1 + 1 0 1 0	

```
<220>
<223>
       amplification primer
<400>
       15
                                                                         23
cttcatcatc gagatagctg tcg
<210>
       16
<211>
       25
<212>
       PRT
<213>
       T. aggregans
<400>
       16
Glu Tyr Arg Arg Leu Arg Thr Thr Tyr Leu Gly Gly Tyr Val Lys
                                      10
Glu Pro Glu Arg Gly Leu Trp Glu Asn
            20
(i)
<210>
       17
<211>
       25
⟨212>
       PRT
<213>
       T. litoralis
m
       17
<400>
Clu Tyr Lys Arg Arg Leu Arg Thr Thr Tyr Leu Gly Gly Tyr Val Lys
£',}
Glu Pro Glu Lys Gly Leu Trp Glu Asn
į.i.
       18
<210>
<211>
       24
       PRT
<212>
<213>
       T. fumicolans
<400>
       18
Glu Leu Glu Arg Arg Gly Gly Tyr Ala Gly Gly Tyr Val Lys Glu
                                      10
Pro Glu Arg Gly Leu Trp Glu Asn
            20
<210>
       19
```

Page 5

```
<211>
       24
<212>
       PRT
<213>
       T. spec. 9N7
<400>
       19
Glu Leu Ala Arg Arg Gly Gly Tyr Ala Gly Gly Tyr Val Lys Glu
1
                                     10
Arg Glu Arg Gly Leu Trp Glu Asn
            20
<210>
       20
<211>
       24
<212>
       PRT
<213>
       T. gorgonarius
<400>
       20
Ghu Leu Ala Arg Arg Glu Ser Tyr Ala Gly Gly Tyr Val Lys Glu
145
                5
                                     10
                                                          15
٢ú
Pro Glu Arg Gly Leu Trp Glu Asn
            20
į.
<210>
       21
<211>
       24
€212>
       PRT
<213>
       P. spec. KOD
Lij
<400>
       21
@u Leu Ala Arg Arg Gln Ser Tyr Glu Gly Gly Tyr Val Lys Glu
                5
                                     10
Pro Glu Arg Gly Leu Trp Glu Asn
            20
<210>
       22
<211>
       25
<212>
       PRT
       P. abysii
<213>
<400>
       22
Glu Tyr Glu Arg Arg Leu Arg Glu Ser Tyr Glu Gly Gly Tyr Val Lys
                                     10
                                                       . 15
```

```
5328us.ST25.txt
Glu Pro Glu Lys Gly Leu Trp Glu Asn
            20
<210>
       23
<211>
       25
<212>
       PRT
<213>
       P. furiosus
<400>
       23
Glu Tyr Gln Arg Arg Leu Arg Glu Ser Tyr Thr Gly Gly Phe Val Lys
Glu Pro Glu Lys Gly Leu Trp Glu Asn
<210>
       24
<211>
       25
<212>
       PRT
<213>
       P. horikoshii
       24
[]
GÎu Tyr Glu Arg Arg Leu Arg Glu Ser Tyr Glu Gly Gly Tyr Val Lys
Glu Pro Glu Lys Gly Leu Trp Glu Asn
20
25
       25
       25
       PRT
       M. jannaschii
<400>
       25
Glu Tyr Arg Arg Arg Val Leu Thr Thr Tyr Glu Gly Gly Tyr Val Lys
Glu Pro Glu Lys Gly Met Phe Glu Asp
            20
                                  25
<210>
       26
```

<211> 25

<212> PRT ·

<213> M. voltae

<400> 26

Ser Tyr Arg Glu Arg Ala Lys Phe Ser Tyr Glu Gly Gly Tyr Val Arg 10 15 Glu Pro Leu Lys Gly Ile Gln Glu Asn 20 25 <210> 27 <211> 25 <212> PRT <213> S. solfataricus <400> 27 Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly Ala Val Val Ile 5 10 15 Asp Pro Pro Ala Gly Ile Phe Phe Asn 20 25 **2**10> 28 211> 212> 25 PRT S. acidocaldarius **4**00> 28 Thr Ala Ala Val Ile Lys Gly Lys Lys Tyr Lys Gly Ala Val Ile

5 10 15 Asp Pro Pro Ala Gly Val Tyr Phe Asn 20 25 <210> 29 25 <212> PRT <213> P. islandicum <400> 29 Thr Lys Ala Ile Ile Lys Gly Lys Lys Tyr Ala Gly Ala Val Leu 5 15 Asp Pro Pro Leu Gly Ile Phe Phe Asn 20 25 <210> 30

Page 8

<211>

25

```
<212>
                                PRT
<213>
                                P. occultum
<400>
                                30
Ser Glu Ala Leu Ile Lys Gly Lys Lys Tyr Gln Gly Ala Leu Val Leu
Asp Pro Pro Ser Gly Ile Tyr Phe Asn
<210>
                                 31
<211>
                                25
<212>
                                PRT
<213>
                                A. pernix
<400>
                                31
Val Gly Ala Ile Ile Lys Asp Lys Lys Tyr Arg Gly Ala Ile Val Leu
Asp Pro Pro Val Gly Ile Phe Phe Arg
  []
 <210>
                                32
<210 / 211 / 22 / 12 / 213 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2100 / 2
                                25
                                PRT
                                S. chwakuensis
                                32
Thr Ala Ala Ile Ser Lys Gly Lys Arg Tyr Lys Gly Ala Val Ile
Asp Pro Pro Ala Gly Val Phe Phe Asn
                                                        20
                                                                                                                                                     25
<210>
                                 33
<211>
                                2325
<212>
                                DNA
<213>
                                T. aggregans
<220>
<221>
                                CDS
<222>
                                (1)..(2325)
<400>
atg ata ttt gac act gac tac ata aca aag gac ggt aaa ccc ata att
```

_	Ile	Phe	Asp	Thr	Asp	Tyr		328us Thr				Lys	Pro	Ile 15	Ile		
_			_		-			gaa Glu 25	ttt			-		gat			96
			ccc					ctt Leu					tcc			:	144
_				-				gag Glu	_						-	-	192
-	_	_	_			_	_	aag Lys			_		_	-	-	2	240
								cat His								2	288
								gct Ala 105									336
- #				-	_	_		ctc Leu		-	_		_			`;	384
								cta Leu								2	432
					-			Gīλ āāā	_					_		2	480
-		_	_		_		_	agg Arg	_				_				528
								tcc Ser 185								į.	576
cgg	ttt	gtg	caa	att	gtc	agg	gaa	aaa Pa	gac age 1		gat	gtc	ctg	ata	act	(	624

Arg	Phe	Val 195	Gln	Ile	Val	Arg		Lys				Val 205	Leu	Ile	Thr		
								ccg Pro								·	672
								ggg Gly									720
								agc Ser									768
_				_			_	gtt Val 265			_						816
								tat Tyr									864
2:2	-	_	_				-	atc Ile	_	_			_				912
_	_	_	_	-	_	_	_	tac Tyr	_	_	_	_	-		_		960
								ttc Phe									1008
								gac Asp 345									1056
								agg Arg									1104
								gaa Glu									1152
act	act	tac	ctg	gga	gga	tac	gta	aaa Pa	gag age 1	_	gaa	aga	ggc	tta	tgg		1200

								328us						_	<u>.</u>	
Thr 385	Thr	Tyr	Leu	Gly	Gly 390	Tyr	Val	Lys	Glu	Pro 395	Glu	Arg	Gly	Leu	Trp 400	
						_		agg Arg	_							1248
-				_			_	act Thr 425		-	_	_		_	_	1296
		_	_	_			_	ggt Gly		_		_				1344
								ggg Gly	_				_			1392
								aca Thr								1440
E-:		_				_	_	gtt Val		-		_		_		1488
								gcg Ala 505								1536
gec								agg Arg								1584
								ttt Phe	_				_	_		1632
_				_			_	gga Gly	_			_				1680
_		_	_	_				tac Tyr								1728
ctg	ctc	gag	ctt	gag	tat	gag	ggc	ttt Pá	tac age 1	-	aga	gga	ttt	ttc	gtc	1776

							50	328us	s.ST2	25.tz	ĸt					
Leu	Leu	Glu	Leu 580	Glu	Tyr	Glu	Gly	Phe 585	Tyr	Leu	Arg	Gly	Phe 590	Phe	Val	
_	_	_	_			_		_	_	_	ggt Gly			_		1824
											gaa Glu 620					1872
											gaa Glu					1920
											gag Glu					1968
****	_	_		_	_		_				cag Gln			_	_	2016
dţa											gca Ala					2064
	_	-	_					_			acg Thr 700			_		2112
											agg Arg					2160
			_	_				_		-	ccc Pro	_	•			2208
											ctt Leu					2256
	_		_	-					_		aaa Lys	_	_		_	2304
gac	gcg	tgg	ctt	aag	aag	tag										2325

# Asp Ala Trp Leu Lys Lys 770

<210> 34

<211> 774

<212> PRT

<213> T. aggregans

<400> 34

Met Ile Phe Asp Thr Asp Tyr Ile Thr Lys Asp Gly Lys Pro Ile Ile 1 5 10 15

Arg Ile Phe Lys Lys Glu Asn Gly Glu Phe Lys Ile Glu Leu Asp Pro 20 25 30

His Phe Gln Pro Tyr Ile Tyr Ala Leu Leu Lys Asp Asp Ser Ala Ile 35 40 45

. T. T.

Asp Glu Ile Lys Ala Ile Lys Gly Glu Arg His Gly Lys Ile Val Arg

drill gray

Val Val Asp Ala Val Lys Val Lys Lys Phe Leu Gly Arg Asp Val

Gla Val Trp Lys Leu Ile Phe Glu His Pro Gln Asp Val Pro Ala Leu

85
90
95

Arg Gly Lys Ile Arg Glu His Pro Ala Val Ile Asp Ile Tyr Glu Tyr 100 105 110

Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 120 125

Met Glu Gly Asp Glu Glu Leu Lys Leu Met Ala Phe Asp Ile Glu Thr 130 135 140

Phe Tyr His Glu Gly Asp Glu Phe Gly Lys Gly Glu Ile Ile Met Ile Page 14

Ser Tyr Ala Asp Glu Glu Glu Ala Arg Val Ile Thr Trp Lys Asn Ile Asp Leu Pro Tyr Val Asp Val Val Ser Asn Glu Arg Glu Met Ile Lys Arg Phe Val Gli Ile Val Arg Glu Lys Asp Pro Asp Val Leu Ile Thr Tyr Asn Gly Asp Asn Phe Asp Leu Pro Tyr Leu Ile Lys Arg Ala Glu া Leu Gly Val Thr Leu Leu Leu Gly Arg Asp Lys Glu His Pro Glu 2.25 . Cij Piro Lys Ile His Arg Met Gly Asp Ser Phe Ala Val Glu Ile Lys Gly į. Arg Ile His Phe Asp Leu Phe Pro Val Val Arg Arg Thr Ile Asn Leu C.J Ļij Pro Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Val Leu Gly Lys Thr į.i. Lys Ser Lys Leu Gly Ala Glu Glu Ile Ala Ala Ile Trp Glu Thr Glu Glu Ser Met Lys Lys Leu Ala Gln Tyr Ser Met Glu Asp Ala Arg Ala Thr Tyr Glu Leu Gly Lys Glu Phe Phe Pro Met Glu Ala Glu Leu Ala Lys Leu Ile Gly Gln Ser Val Trp Asp Val Ser Arg Ser Ser Thr Gly Page 15

350

Asn Leu Val Glu Trp Tyr Leu Leu Arg Val Ala Tyr Glu Arg Asn Glu 355 360 365

340

Ľ.

μī

IJ

Leu Ala Pro Asn Lys Pro Asp Glu Glu Glu Tyr Arg Arg Arg Leu Arg 370 375 380

Thr Thr Tyr Leu Gly Gly Tyr Val Lys Glu Pro Glu Arg Gly Leu Trp 385 390 395 400

Glu Asn Ile Thr Tyr Leu Asp Phe Arg Cys Leu Tyr Pro Ser Ile Ile 405 410 415

Val Thr His Asn Val Ser Pro Asp Thr Leu Glu Arg Glu Gly Cys Lys
420
430

Asin Tyr Asp Val Ala Pro Ile Val Gly Tyr Lys Phe Cys Lys Asp Phe 435 440 445

Pro Gly Phe Ile Pro Ser Ile Leu Gly Glu Leu Ile Thr Met Arg Gln 450 460

Glu Ile Lys Lys Met Lys Ala Thr Ile Asp Pro Ile Glu Lys Lys 465 470 475 480

Met Leu Asp Tyr Arg Gln Arg Ala Val Lys Leu His Ala Asn Ser Tyr 485 490 495

Tyr Gly Tyr Met Gly Tyr Pro Lys Ala Arg Trp Tyr Ser Lys Glu Cys
500 505 510

Ala Glu Ser Val Thr Ala Trp Gly Arg His Tyr Ile Glu Met Thr Ile 515 520 525

Lys Glu Ile Glu Glu Lys Phe Gly Phe Lys Val Leu Tyr Ala Asp Thr
Page 16

Asp 545	Gly	Phe	Tyr	Ala	Thr 550	Ile	Pro	Gly	Glu	Lys 555	Pro	Glu	Thr	Ile	Lys 560
Lys	Lys	Ala	Lys	Glu 565	Phe	Leu	Lys	Tyr	Ile 570	Asn	Ser	Lys	Leu	Pro 575	
Leu	Leu	Glu	Leu 580	Glu	Tyr	Glu	Gly	Phe 585	Tyr	Leu	Arg	Gly	Phe 590	Phe	Val
Ala	Lys	Lys 595	Arg	Tyr	Ala	Val	Ile 600	Asp	Glu	Glu	Gly	Arg 605	Ile	Thr	Thr
Arg	Gly 610	Leu	Glu	Val	Val	Arg 615	Arg	Asp	Trp	Ser	Glu 620	Ile	Ala	Lys	Glu
: :	Gln	Ala	Lys	Val	Leu 630	Glu	Ala	Ile	Leu	Lys 635	Glu	Asp	Ser	Val	Glu 640
	Ala	Val	Glu	Ile 645	Val	Lys.	Asp	Val	Val 650	Glu	Glu	Ile	Ala	Lys 655	Tyr
	Val	Pro	Leu 660	Glu	Lys	Leu	Val	Ile 665	His	Glu	Gln	Ile	Thr 670	Lys	Asp
Leu	Ser	Glu 675	Tyr	Lys	Ala	Ile	Gly 680	Pro	His	Val	Ala	Ile 685	Ala	Lys	Arg
Leu	Ala 690	Ala	Lys	Gly	Ile	Lys 695	Val	Arg	Pro	Gly	Thr 700	Ile	Ile	Ser	Tyr
Ile 705	Val	Leu	Arg	Gly	Ser 710	Gly	Lys	Ile	Ser	Asp 715	Arg	Val	Ile	Leu	Leu 720
Ser	Glu	Tyr	Asp	Pro	Lys	Lys	His	_	Tyr age :	_	Pro	Asp	Tyr	Tyr	Ile

725

#### 5328us.ST25.txt 730

735

Glu Asn Gln Val Leu Pro Ala Val Leu Arg Ile Leu Glu Ala Phe Gly .740 745 750

Tyr Arg Lys Glu Asp Leu Lys Tyr Gln Ser Ser Lys Gln Val Gly Leu 755 760 765

Asp Ala Trp Leu Lys Lys 770